EXTENDED REPORT

Clinical utility of the anti-CCP assay in patients with rheumatic diseases

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Objectives: To determine the frequency of antibodies to cyclic citrullinated peptides (CCP) in a group of patients with a diversity of rheumatic diseases.

Methods: 249 consecutive sera from an arthritis clinic sent for rheumatology testing were selected for testing with the anti-CCP2 assays and for the presence of rheumatoid factor (RF). Patient charts were reviewed for demographic information, clinical diagnosis, radiographic information, and other laboratory data.

Results: The sensitivity and specificity of anti-CCP reactivity for the diagnosis of rheumatoid arthritis (RA) were 66.0% and 90.4%, respectively. This compared with the sensitivity and specificity of RF for RA at 71.6% and 80.3%. Furthermore, 10/29 (34%) RF– patients with RA demonstrated reactivity to CCP. The presence of *either* anti-CCP or RF increased testing sensitivity for diagnosis of RA to 81.4%; the presence of *both* RF and anti-CCP demonstrated a testing specificity similar to that of anti-CCP reactivity alone for the diagnosis of RA (91.1%).

Conclusions: The detection of anti-CCP is useful for the diagnosis of RA, in fact even more so than RF, because of its higher specificity.

Rheumatoid arthritis (RA) is a common rheumatic disease of uncertain aetiology with a significant level of morbidity. Despite decades of study and the development of a series of classification criteria,[1] the diagnosis of RA remains empirical and imprecise, particularly early in the course of disease. Because early initiation of disease modifying treatments can significantly improve long term outcomes for patients with RA, there is considerable motivation to accurately diagnose RA in patients with inflammatory arthritis early in the course of disease.[2 3] The ability to identify those patients who will have progressive, erosive disease also remains an objective because this subset may benefit most from early aggressive treatment. Currently, most clinical specialists rely on a combination of clinical acumen derived from accumulated experience and objective laboratory and radiological studies to make a diagnosis, to predict disease course, and to guide treatment.

Serological studies form a cornerstone of laboratory based patient assessment in rheumatology. The presence of "rheumatoid factor" (RF) was identified in patients with RA over 50 years ago[4]; assays for RF remain one of the American College of Rheumatology (ACR) classification criteria for RA. The RF assay, in its current manifestation, remains suboptimal as a diagnostic test, as it lacks sensitivity (54–88%) and specificity (48–92%)[5–9]; it is present frequently in many other disease states[10–12] (reviewed by Shmerling and Delbanco,[13] Carson,[14] and Bridges[15]), and its incidence increases with age.[10 13] Although RF significantly predicts worse outcome from both functional status and radiographic joint destruction standpoints,[16–22] there is substantial room for improvement in predicting disease severity.

The shortcomings of the RF assay have provided impetus for identification of other serological assays for RA. This search has yielded serological reactivity to a number of autoantigens in subsets of patients with RA, including antikeratin antibodies (AKA),[23] Sa,[24] BiP,[25 26] RA33,[27] glucose-6-phosphate isomerase,[28–30] and antiperinuclear factor (APF or antifillagrin)[31](reviewed by van Boekel *et al*[32]). Although these autoantibodies have all demonstrated lower sensitivity for diagnosis of RA than the RF, many of them are present almost exclusively in patients with RA. Analysis of AKA and APF autoantibodies showed that most of the reactivity present against these antigens was directed against citrulline residues, a post-translational modification of the amino acid arginine.[33] This discovery led to the development of assays employing cyclic citrullinated peptides (CCP) to measure antibodies recognising citrullinated antigens as a diagnostic test for RA. Initial studies characterising the frequency of antibodies to CCP in mixed cohorts containing patients with rheumatic diseases, infectious diseases, and healthy patients, have shown it to be moderately sensitive (68%) but highly specific (98%) for RA.[9] Furthermore, analyses of the predictive value of CCP for RA in early inflammatory arthritis and the predictive value for functional status and radiographic erosions have suggested significant correlations.[8 21 22] Indeed, multiple regression analysis has suggested the importance of anti-CCP in predicting both persistent *v* self limited arthritis and erosive *v* non-erosive disease.[18]

Although several studies have assessed the anti-CCP assay in RA, for many of these studies a significant fraction of control sera were derived from a "normal" cohort; the discriminative functional characteristics of this assay remain largely unproved when surveyed in a cohort of patients with a diversity of rheumatic diseases. Because the operating utility of this assay resides in distinguishing RA from other rheumatic disorders, we sought to assess the anti-CCP assay in a group of patients with a variety of these diseases.

Abbreviations: ACR, American College of Rheumatology; AKA, antikeratin antibodies; APF, antiperinuclear factor; CCP, cyclic citrullinated peptides; IF, immunofluorescent; JRA, juvenile rheumatoid arthritis; OA, osteoarthritis; NPV, negative predictive value; PPV, positive predictive value; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; ROC, receiver operating characteristic; SLE, systemic lupus erythematosus; SSC, sensitised sheep cell

Table 1 Patient demographics by diagnosis group

Patient group	Number of patients	Age, years Mean (range)	Number (%) Female
RA	103	55.4 (24–86)	87 (84)
JRA	21	30.9 (15–50)	18 (86)
PsA	21	44.6 (24–70)	15 (71)
Spondylitis	11	39.0 (26–54)	4 (36)
Inflammatory arthritis	26	46.2 (16–77)	15 (58)
SLE	39	37.7 (18–61)	36 (92)
Non-inflammatory	23	49.6 (19–82)	19 (83)
Other inflammatory condition	5	59.2 (49–80)	3 (60)

METHODS

Serum samples

Two hundred and forty nine unique consecutive serum samples sent from the BWH Arthritis Center to the BWH Clinical Immunology Laboratory for rheumatology testing were selected for further analysis. Patient charts were reviewed for demographic information, clinical diagnosis, radiographic information, and other laboratory data. Rheumatic diagnoses were established by diagnosis of the attending rheumatologist and/or by review of laboratory, radiological, and clinic notes, applying ACR classification criteria. In this cohort, 226 patients had inflammatory disease (RA, n=103; systemic lupus erythematosus (SLE), n=39; psoriatic arthritis (PsA), n=21; juvenile rheumatoid arthritis (JRA), n=21; "inflammatory arthritis", n=26; spondylitis, n=11; other, n=5) and 23 patients had non-inflammatory disease (osteoarthritis (OA), n=10; fibromyalgia, n=10, mechanical pain, n=2, arthralgia, n=1). One hundred and ninety seven (79%) of these patients were female with a wide variation in age (18–86 years) (table 1).

Radiographic analysis

Radiographic identification of joint erosions was investigated in the subsets of patients diagnosed with RA, PsA, JRA, and inflammatory arthritis. Joint radiographs were available for 129/171 patients. All radiographic diagnoses were abstracted from formal interpretation by an attending radiologist.

Table 2 Sensitivity and specificity of anti-CCP and RF for presence of rheumatoid arthritis (RA). CCP2 (n=249); RF (n=214)

	Sensitivity (%)	Specificity (%)	PPV	NPV
CCP	66.0	90.4	82.9	79.0
RF	71.6	80.3	76.8	75.2
CCP or RF	81.4	79.5	78.3	82.4
CCP and RF	56.9	91.1	85.3	69.9

Table 3 Comparison of anti-CCP and RF reactivity

	Patients with RA No (%)	Other patients No (%)
CCP (+)	(n=68)	(n=14)
RF +	58 (85)	10/11* (91)
RF –	10 (15)	1/11* (9)
CCP (–)	(n=35)	(n=132)
RF +	15/34* (44)	12/101* (12)
RF –	19/34* (56)	89/101* (88)

*RF analysis performed on 214 of 249 samples.

Data measurement and analysis

CCP measurement: anti-CCP activity was determined by an enzyme linked immunosorbent assay (ELISA) using a commercial anti-CCP2 assay provided by the Axis-Shield Corp. *Rheumatoid factor measurement*: total RF was determined by nephelometry on 214 of the 249 patients in this study; 35 samples contained insufficient volume to measure RF. Receiver operating characteristic (ROC) curves were generated by the method of Metz.[34] Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated as described.[35] Best fit curves were generated by using non-linear regression calculations.

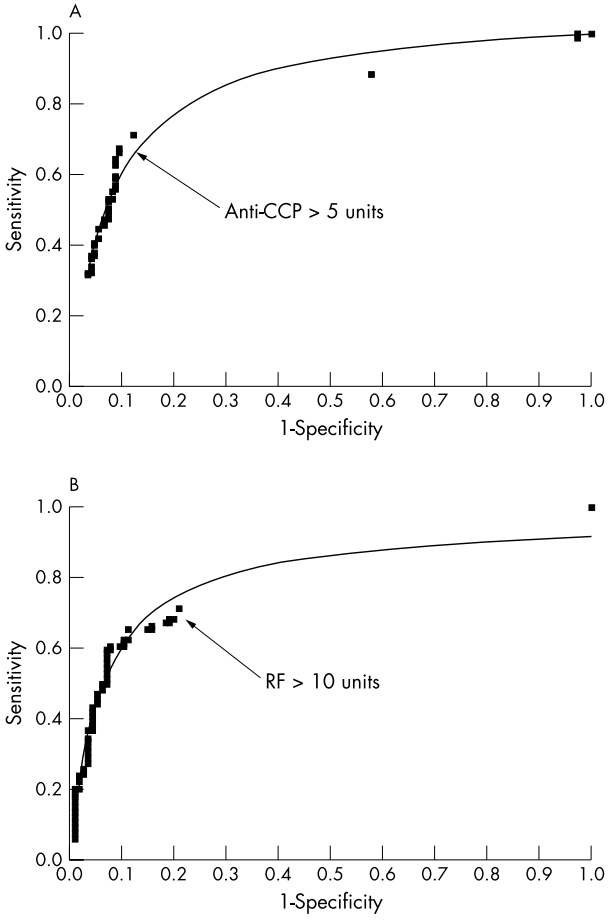

Figure 1 ROC curves for anti-CCP (A) and RF (B) assays. Individual datapoints are represented as small squares. A best fit curve was generated by non-linear regression calculation. Arrows mark the cut off values used for this study.

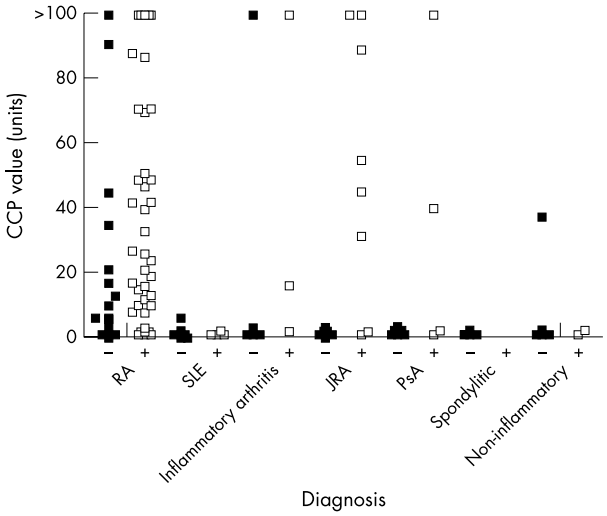

Figure 2 CCP reactivity in rheumatic disease subsets. Shown are the levels of anti-CCP reactivity in sera from patients with labelled rheumatic diagnoses. Closed squares RF–; open squares RF+.

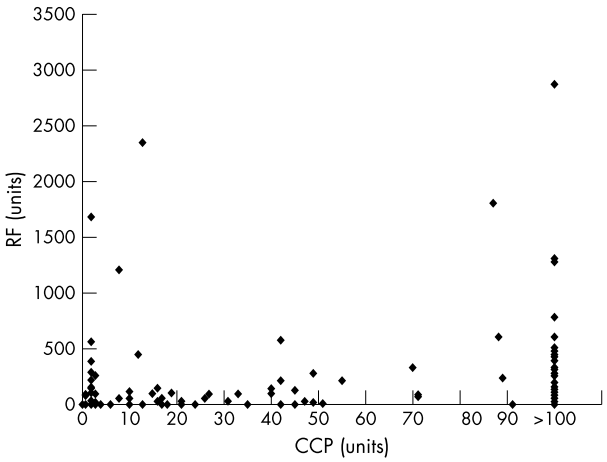

Figure 3 RF *v* CCP values. The values in units of RF and CCP activity in 214 rheumatic disease patients are shown. Correlation coefficient (*R*)=0.34.

RESULTS

CCP correlation with RA

In this cohort of 249 patients dominated by rheumatic disease (table 1), 82/249 samples tested positive for anti-CCP activity at >5 units reactivity. Of these 82 patients, 68 had RA. This translates into a sensitivity and specificity of anti-CCP reactivity for the diagnosis of RA of 66.0% and 90.4%, respectively (table 2). This compared with the sensitivity and specificity of RF for RA at 71.6% and 80.3% (table 2). In the RA cohort, 58/68 (85%) CCP+ patients were also RF+. These tests also had independent reactivity in a significant subset of patients: 10/29 (34%) patients with RA who were RF– showed reactivity to CCP and 15/34 (44%) CCP– patients with RA showed reactivity to RF (table 3).

To determine the diagnostic characteristics of the anti-CCP and RF assays in our rheumatic disease cohort we determined both the relation between sensitivity and specificity at different test cut off values (displayed graphically in our ROC plots (fig 1)) and the positive and negative predictive values of these assays (table 2). These analyses confirmed the optimal cut off value for CCP (anti-CCP >5 units). There were two apparent inflection points in the RF analysis, one at RF>10 (our cut off value) and another at the higher cut off value of RF>22.

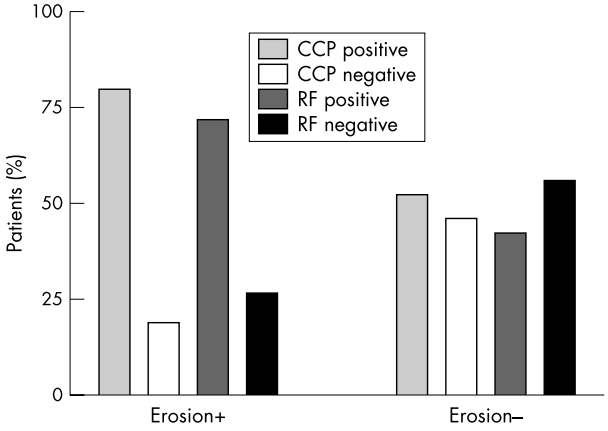

Figure 4 Correlation of anti-CCP activity and radiographic joint destruction. The percentage of patients with anti-CCP reactivity and RF relative to the presence of erosions in patients with available radiographs (n=129) is shown.

We also examined the utility of combining the RF and anti-CCP diagnostic tests at optimal test performance values. Allowing the presence of either autoantibody (either RF or anti-CCP) increased the sensitivity for detecting RA to 81.4% (table 2) without substantially altering the specificity for RA (79.5%) from that of RF alone. Conversely, requiring the presence of both autoantibodies (RF and anti-CCP positivity) decreased the sensitivity for diagnosis of RA to 56.9% without demonstrating a substantial increase in specificity (91.1%) relative to that of anti-CCP reactivity alone (90.4%).

CCP reactivity in rheumatic disease subsets

Although the specificity of anti-CCP for RA in our cohort was 90.4%, we sought to delineate the presence of anti-CCP activity in other rheumatic conditions. Of the 14 anti-CCP+ patients without RA in this cohort, 13 had another inflammatory disease (JRA, n=6; inflammatory arthritis, n=3; other, n=4) and only one had a non-inflammatory disease (fibromyalgia); most "false positives" were accounted for by the JRA subset of patients (fig 2). With the exception of the JRA cohort, there was virtually no anti-CCP reactivity in serum from patients with PsA (2/21), SLE (1/39), spondylitic variants (0/11), or inflammatory arthritis (3/26) (fig 2). It should be noted that our JRA cohort comprised adults (average age 31) with longstanding disease (average disease duration 21 years) and high prevalence of erosions (79%).

Correlation of RF and CCP reactivity

Knowing there existed a substantial correspondence of reactivity between the RF and CCP assays, we sought to determine if levels of reactivity correlated between these tests. In a comparison of levels of anti-CCP and RF activity, we found no substantial correlation (R=0.34) (fig 3).

Correlation of anti-CCP reactivity with joint erosions

We assessed the correlation between anti-CCP activity and radiographic erosions for patients with radiographs in both the RA subset and the entire anti-CCP(+) groups of patients (table 4, fig 4). In the entire cohort with radiographs, 63% of patients with erosions demonstrated serum anti-CCP reactivity while 65% of patients without erosions lacked anti-CCP reactivity. For the RA patient subset, 72% of those with anti-CCP activity displayed evidence of radiographic erosions. Of the patients with RA with erosions, 81% demonstrated anti-CCP reactivity. However, a substantial fraction of patients with RA without erosions also demonstrated anti-CCP reactivity (53%).

Table 4 Correlation of anti-CCP activity radiographic joint destruction. Shown is the subset analysis of anti-CCP reactivity and radiographic presence of erosion in patients with rheumatoid arthritis (RA), juvenile rheumatoid arthritis (JRA), psoriatic arthritis (PsA), and inflammatory arthritis. (No (%))

	RA (n=82)	PsA (n=16)	JRA (n=19)	Inflammatory arthritis (n=12)
Erosion +	(n=52)	(n=5)	(n=15)	(n=6)
CCP +	42 (81)	1 (20)	6 (40)	0 (0)
CCP –	10 (19)	4 (80)	9 (60)	6 (100)
Erosion –	(n=30)	(n=11)	(n=4)	(n=6)
CCP +	16 (53)	1 (9)	0 (0)	1 (17)
CCP –	14 (47)	10 (91)	4 (100)	5 (83)

Extending our analysis to include JRA, PsA, and inflammatory arthritis we found no correlation between anti-CCP reactivity and radiographic joint destruction for inflammatory arthritis (0/6) and PsA (1/5) (table 4). In the 19 patients with JRA with radiographs, although only 40% of those with erosions demonstrated anti-CCP activity, all patients with anti-CCP reactivity demonstrated erosions.

DISCUSSION

Historically, the use of RF as a diagnostic tool for RA has been and remains problematic. After an initially serendipitous recognition that antibodies to IgG were often found in high titre in patients with RA,[4 11] the sensitised sheep cell (SSC) assay was developed. This assay, cumbersome to perform, was positive in about 60% of patients with RA and infrequently in normal subjects or patients with other rheumatic diseases,[11] and acquired the designation "rheumatoid factor" (RF). This test soon helped to classify patients into "seropositive *v* seronegative" arthritis. However, shortcomings of the SSC assay led to the development of an assay dependent upon RF anti-Ig activity agglutinating IgG coated latex particles—the latex fixation assay. The latex fixation assay, easier to perform and more reproducible than the SSC assay, increased the sensitivity for RA to about 70–90% in most series. Unfortunately, the latex fixation assay lacks specificity, being positive in many patients with various chronic disease states[10–12] (reviewed by Shmerling and Delbanco,[13] Carson,[14] and Bridges[15]). Although nephelometry, which also detected IgM anti-IgG RF, was technically more reproducible and easier to perform, it did not improve sensitivity (82%) or specificity (92%) for RA relative to latex agglutination.[5]

Concurrently, other autoantibodies have been found in patients with RA who were tested for antinuclear antibodies by the immunofluorescent (IF) technique. These assays are referred to as the APF and AKA because of their anatomical location on IF.[23 31] When present, they demonstrate high specificity (88–99%) for a diagnosis of RA.[6 23 36] However, because these assays have low sensitivity (~50%) and are cumbersome to perform, their clinical application remains limited. Subsequent characterisation demonstrated that much of the reactivity to these autoantigens was contained in citrulline containing regions of the antigens.[33] Antibodies to citrullinated proteins can be detected by enzyme immunoassay, which is much more reproducible and easier to perform than the IF assays for perinuclear factor. Initial studies using citrullinated peptide as substrate demonstrated a sensitivity of 76% and a specificity of 96% for RA.[33] Subsequently, a modified assay was developed using CCP.[9] This assay detected IgG antibodies to CCP in 68% of patients with RA. Although it had a somewhat lower sensitivity than the RF test, the specificity of anti-CCP for RA in that population was 96%—better than that previously reported in the RF test for RA (48–92%).[5–9] This represented a great clinical diagnostic improvement.

Subsequent studies have confirmed the highly specific nature of anti-CCP activity in patients with RA and correlated the presence of anti-CCP with erosive disease.[8 21 22] Furthermore, inclusion of anti-CCP activity in disease models predicting persistent and erosive disease significantly improved the performance of these models.[18]

Our experience with the anti-CCP assay in 249 patients with rheumatic diseases indicates a sensitivity and specificity for RA of 66% and 90.4% (not in comparison with normal subjects but in comparison with patients with other rheumatic diseases). This high sensitivity and specificity in our hands confirms the initial experience of others. In addition, we observed a low frequency of anti-CCP in other rheumatic diseases including SLE, inflammatory arthritis, PsA, spondylitic variants, OA, and fibromyalgia. Of particular interest was the fact that only 3/26 patients with "inflammatory arthritis" (clinically felt to be distinct from RA) were positive. Whether this lack of reactivity is of prognostic value, as noted by Visser *et al*,[18] will be of interest in continuing analysis.

Another interesting observation was in JRA: 6/21 had anti-CCP reactivity. This cohort of adult patients with JRA, with an average duration of disease of 21 years (range 6–36), was the only group outside of RA that had a significantly increased frequency of anti-CCP. In JRA, all anti-CCP+ patients were RF+ and had erosive disease, although the majority of patients with erosive disease had no anti-CCP reactivity. Whether these findings portend common pathogenic mechanisms within these subsets of patients with anti-CCP reactivity remains an interesting speculation.

Where does this leave us with respect to finding a laboratory test specific and sensitive for the diagnosis of RA? In our cohort, designed to model "real life" clinical use of this assay, anti-CCP certainly brings us closer than we were with RF, particularly from the vantage of specificity. The low "false positive" rate in inflammatory arthritis groups other than RA (excluding chronic JRA) significantly increases the usefulness of anti-CCP. From a practical perspective, it would be useful to perform the RF and anti-CCP assays concurrently. In our hands, performing both assays and allowing a positive result in either assay (either RF or anti-CCP) confers higher sensitivity for RA (81.4%). Furthermore, both RF and anti-CCP are moderately strongly associated with articular erosions, suggesting that they reflect in some way the severity and progression of RA. Therefore we conclude that detection of anti-CCP is very useful for the diagnosis of RA, in fact even more so than RF, because of its higher specificity. Preliminary observations also suggest that the combination of testing for both RF and anti-CCP may be even more useful.

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